

# An Analysis of a Circadian Model Using The Small-Gain Approach to Monotone Systems

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## Abstract

In this note, we show how certain properties of Goldbeter's 1995 model for circadian oscillations can be proved mathematically, using techniques from the recently developed theory of monotone systems with inputs and outputs. The theory establishes global asymptotic stability, and in particular no oscillations, if the rate of transcription is somewhat smaller than that assumed by Goldbeter. This stability persists even under arbitrary delays in the feedback loop.

## 1 Introduction

The molecular biology underlying the circadian rhythm in *Drosophila* is the focus of a large amount of both experimental and theoretical work. Goldbeter proposed a simple model for circadian oscillations in [4] (see also his book [5]). Although by now several more realistic models are available, in particular incorporating other genes, this simpler model exhibits many realistic features, such as a 24-hour period. The key to the model is the inhibition of *per* gene transcription by its protein product PER, forming an autoregulatory negative feedback loop.

In this note, we show how certain properties of the model can be proved mathematically, using techniques from the recently developed theory of monotone systems with inputs and outputs. The theory establishes global asymptotic

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stability, and in particular no oscillations, if the rate of transcription is somewhat smaller than that assumed by Goldbeter. This stability persists even under arbitrary delays in the negative feedback loop. On the other hand, a larger – but still smaller than Goldbeter’s – strength, in the presence of delays, results in oscillations.

The terminology and notations are as given in [2, 3], and are not repeated here.

## 2 The Model

The model is as shown in Figure 1. PER protein is synthesized at a rate pro-

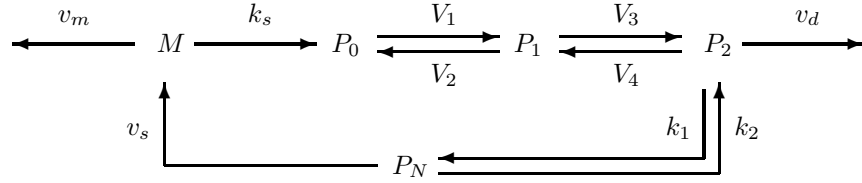


Figure 1: Goldbeter’s Model

portional to its mRNA concentration. Two phosphorylation sites are available, and constitutive phosphorylation and dephosphorylation occur with saturation dynamics, at maximum rate  $v_i$ ’s and with Michaelis constants  $K_i$ . Doubly phosphorylated PER is degraded, also satisfying saturation dynamics (with parameters  $v_d, k_d$ ), and it is translocated to the nucleus with rate constant  $k_1$ . Nuclear PER inhibits transcription of the *per* gene, with a Hill-type reaction of cooperativity degree  $n$  and threshold constant  $K_I$ , and mRNA is produced. and translocated to the cytoplasm, at a rate determined by a constant  $v_s$ . Additionally, there is saturated degradation of mRNA (constants  $v_m$  and  $k_m$ ).

The equations for concentrations are as follows:

$$\begin{aligned}
 \dot{M} &= v_s K_I^n / (K_I^n + P_N^n) - v_m M / (k_m + M) \\
 \dot{P}_0 &= k_s M - V_1 P_0 / (K_1 + P_0) + V_2 P_1 / (K_2 + P_1) \\
 \dot{P}_1 &= V_1 P_0 / (K_1 + P_0) - V_2 P_1 / (K_2 + P_1) - V_3 P_1 / (K_3 + P_1) + V_4 P_2 / (K_4 + P_2) \\
 \dot{P}_2 &= V_3 P_1 / (K_3 + P_1) - V_4 P_2 / (K_4 + P_2) - k_1 P_2 + k_2 P_N - v_d P_2 / (k_d + P_2) \\
 \dot{P}_N &= k_1 P_2 - k_2 P_N
 \end{aligned}$$

where the subscript  $i = 0, 1, 2$  in the concentration  $P_i$  indicates the degree of phosphorylation of PER protein,  $P_N$  is used to indicate the concentration of PER in the nucleus, and  $M$  indicates the concentration of *per* mRNA. The parameters (in suitable units  $\mu M$  or  $h^{-1}$ ) are as in the following table:

Parameter	Value	Parameter	Value
$k_2$	1.3	$k_1$	1.9
$V_1$	3.2	$V_2$	1.58
$V_3$	5	$V_4$	2.5
$v_s$	0.76	$k_m$	0.5
$k_s$	0.38	$v_d$	0.95
$k_d$	0.2	$n$	4
$K_1$	2	$K_2$	2
$K_3$	2	$K_4$	2
$K_I$	1	$v_m$	0.65

With these parameters, there are limit cycle oscillations. We leave all fixed except  $v_s$ , and show that there are no oscillations if  $v_s = 0.4$ , but oscillations exist if  $v_s = 0.5$  and there are delays in the negative regulatory loop, either in transcription or in translation (or in both).

We choose to view the system as the feedback interconnection of two sub-systems, see Figure 2.

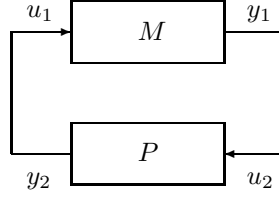


Figure 2: Systems in feedback

### mRNA System

The first ( $M$ ) subsystem is described by the scalar differential equation

$$\dot{M} = v_s K_I^n / (K_I^n + u_1^n) - v_m M / (k_m + M)$$

with input  $u_1$  and output  $y_1 = k_s M$ .

### PER System

The second ( $P$ ) subsystem is four-dimensional:

$$\begin{aligned} \dot{P}_0 &= u_2 - V_1 P_0 / (K_1 + P_0) + V_2 P_1 / (K_2 + P_1) \\ \dot{P}_1 &= V_1 P_0 / (K_1 + P_0) - V_2 P_1 / (K_2 + P_1) - V_3 P_1 / (K_3 + P_1) + V_4 P_2 / (K_4 + P_2) \\ \dot{P}_2 &= V_3 P_1 / (K_3 + P_1) - V_4 P_2 / (K_4 + P_2) - k_1 P_2 + k_2 P_N - v_d P_2 / (k_d + P_2) \\ \dot{P}_N &= k_1 P_2 - k_2 P_N \end{aligned}$$

with input  $u_2$  and output  $y_2 = P_N$ .

Assume from now on that:

$$v_s \leq 0.54 \quad (1)$$

(the remaining parameters will be constrained below, in such a manner that those in the previously given table will satisfy all the constraints).

As state-space for the first system, we will pick a compact interval  $X_1 = [0, \bar{M}]$ , where

$$\frac{v_s k_m}{v_m - v_s} \leq \bar{M} < \frac{v_d}{k_s} \quad (2)$$

and we assume that  $v_s < v_m$ . Note that the first inequality implies that

$$v_s < \frac{v_m \bar{M}}{k_m + \bar{M}} \quad (3)$$

and therefore

$$v_s K_I^n / (K_I^n + u_1^n) - v_m \bar{M} / (k_m + \bar{M}) < 0$$

for all  $u_1 \geq 0$ , so that indeed  $X_1$  is forward-invariant for the dynamics. With the parameters shown in the table given earlier (except for  $v_s$ , which is picked as in (1)),

$$\bar{M} = 2.45$$

satisfies all the constraints. As input space for the mRNA system, we pick  $U_1 = \mathbb{R}_{\geq 0}$ , and as output space  $Y_1 = [0, v_s)$ . Note that  $y_1 = k_s M \leq k_s \bar{M} < v_s$ , by (2), so the output belongs to  $Y_1$ .

For the second system, the state space is  $\mathbb{R}_{\geq 0}^4$ , the input space is  $U_2 = Y_1$ , and the output space is  $Y_2 = U_1$ .

When looking at the first system, we view  $U_1$  as ordered by the cone  $\mathbb{R}_{\leq 0}$ , but  $U_2, Y_1, Y_2$  are all ordered in the usual manner (cone  $\mathbb{R}_{\geq 0}$ ).

### 3 Monotonicity and Characteristics

The first system is monotone, and has a well-defined characteristic, in the sense of [2]. Monotonicity is clear (one-dimensional system), and the existence of characteristics is immediate from the fact that  $\dot{M} > 0$  for  $M < k_1(u_1)$  and  $\dot{M} < 0$  for  $M > k_1(u_1)$ , where, for each constant input  $u_1$ ,

$$k_1(u_1) = \frac{v_s K_I^n k_m}{v_m K_I^n + v_m u_1^n - v_s K_I^n}$$

(which is an element of  $X_1$ ).

Note that all solutions of the differential equations which describe the  $M$ -system, even those that do not start in  $X_1$ , enter  $X_1$  in finite time (because  $\dot{M}(t) < 0$  whenever  $M(t) \geq \bar{M}$ , for any input  $u_1(\cdot)$ ). The restriction to the state space  $X_1$  (instead of using all of  $\mathbb{R}_{\geq 0}$ ) is done for convenience, so that one can view the output of the  $M$  system as in input to the  $P$ -subsystem. (Desirable properties of the  $P$ -subsystem depend on the restriction imposed on  $U_2$ .) Given

any trajectory, its asymptotic behavior is independent on the behavior in an initial finite time interval, so this does not change the conclusions to be drawn. (Note that solutions are defined for all times –no finite explosion times– because the right-hand sides of the equations have linear growth.)

Monotonicity of the second system is also clear, from the fact that  $\frac{\partial \dot{P}_i}{\partial P_j} > 0$  for all  $i \neq j$ ; in fact, this is a *strongly monotone tridiagonal system* ([6, 7]). We show that (for the parameters in the table, as well as for a larger set of parameters) the system has, for each constant input  $u$ , a unique equilibrium, and trajectories are all bounded; it follows then from [6, 7] that the unique equilibrium is globally asymptotically stable, which means that characteristics are well-defined.

**Proposition 3.1** Suppose that the following conditions hold:

- $v_d + V_2 < V_1$
- $V_1 + V_4 < V_2 + V_3$
- $0 \leq c < v_d$
- $V_4 + v_d < V_3$

and that all constants are positive and the input  $u_2(t) \equiv c$ . Then the  $P$ -system has a unique globally asymptotically stable equilibrium.

This will be a corollary of the following more general result.

**Theorem 1** Consider a system of the following form:

$$\begin{aligned}\dot{x}_0 &= c - \alpha_0(x_0) + \beta_0(x_1) \\ \dot{x}_1 &= \alpha_0(x_0) - \beta_0(x_1) - \alpha_1(x_1) + \beta_1(x_2) \\ \dot{x}_2 &= \alpha_1(x_1) - \beta_1(x_2) - \alpha_2(x_2) - \gamma_2(x_2) + \gamma_3(x_3) \\ \dot{x}_3 &= \gamma_2(x_2) - \gamma_3(x_3)\end{aligned}$$

evolving on  $\mathbb{R}_{\geq 0}^4$ , where  $c \geq 0$  is a constant, and the functions

$$\alpha_i, \beta_i, \gamma_i : [0, \infty) \rightarrow [0, \infty)$$

are all differentiable, with derivatives everywhere positive, and so that  $\alpha_i$  and  $\beta_i$  are bounded, for each  $i$ , and  $\gamma_1, \gamma_2$  are unbounded. Furthermore, suppose that the following conditions hold:

$$\alpha_2(\infty) + \beta_0(\infty) < \alpha_0(\infty) \tag{4}$$

$$\alpha_0(\infty) + \beta_1(\infty) < \alpha_1(\infty) + \beta_0(\infty) \tag{5}$$

$$\alpha_2(\infty) + \beta_1(\infty) < \alpha_1(\infty) \tag{6}$$

$$c < \alpha_2(\infty). \tag{7}$$

Then, there is a (unique) globally asymptotically stable equilibrium for the system.

Note that (4) and (7) imply also:

$$c + \beta_0(\infty) < \alpha_0(\infty). \quad (8)$$

*Proof.* We start by noticing that solutions are defined for all  $t \geq 0$ . Consider any maximal solution  $x(t) = (x_0(t), x_1(t), x_2(t), x_3(t))$ . From

$$\frac{d}{dt}(x_0 + x_1 + x_2 + x_3) = c - \alpha_2(x_2) \quad (9)$$

we conclude there is an estimate  $x_i(t) \leq \sum_i x_i(t) \leq \sum_i x_i(0) + tc$  and hence there are no finite escape times. Moreover, we claim that  $x(\cdot)$  is bounded.

Since the system is a strongly monotone tridiagonal system, we know that  $x_3(t)$  is *eventually monotone*. That is, for some  $T > 0$ , either

$$\dot{x}_3(t) \geq 0 \quad \forall t \geq T \quad (10)$$

or

$$\dot{x}_3(t) \leq 0 \quad \forall t \geq T. \quad (11)$$

Hence,  $x_3(t)$  admits a limit, either finite or infinite. Assume first that  $x_3(t) \rightarrow \infty$ . Then, case (11) cannot hold, so (10) holds. Looking at the differential equation for  $x_3$ , we know that  $\gamma_2(x_2(t)) - \gamma_3(x_3(t)) \geq 0$  for all  $t \geq T$ , which means that

$$x_2(t) \geq \gamma_2^{-1}(\gamma_3(x_3(t))) \rightarrow \infty.$$

Looking again at (9), and using that  $c - \alpha_2(\infty) < 0$  (property (7)), we conclude that  $\frac{d}{dt}(x_0 + x_1 + x_2 + x_3)(t) < 0$  for all  $t$  sufficiently large. Thus  $x_0 + x_1 + x_2 + x_3$  is bounded (and nonnegative), and this implies that  $x_2$  is bounded, a contradiction. So  $x_3$  is bounded.

Next we examine the equation for  $\dot{x}_2$ . The two positive terms are bounded: the one involving  $\alpha_1$  because  $\alpha_1$  is a bounded function, and the one involving  $\gamma_3$  because  $x_3$  is bounded. Thus

$$\dot{x}_2 \leq v(t) - \alpha_2(x_2),$$

where  $0 \leq v(t) \leq k$  for some constant  $k$ . Thus  $\dot{x}_2(t) < 0$  whenever  $x_2(t) > \gamma_2^{-1}(k)$ , and this proves that  $x_2$  is bounded, as claimed.

Now we show that  $x_0$  and  $x_1$  are bounded as well. For  $x_0$ , it is enough to notice that  $\dot{x}_0 \leq c - \alpha_0(x_0) + \beta_0(\infty)$ , so that

$$x_0(t) > \alpha_0^{-1}(c + \beta_0(\infty)) \Rightarrow \dot{x}_0(t) < 0$$

so (8) shows that  $x_0$  is bounded. Similarly, for  $x_1$  we have that  $\dot{x}_1 \leq \alpha_0(\infty) - \beta_0(x_1) - \alpha_1(x_1) + \beta_1(\infty)$  so (5) provides boundedness.

Once that boundedness has been established, if we also show that there is a unique equilibrium then the theory of strongly monotone tridiagonal systems ([6, 7]) will ensure global asymptotic stability of the equilibrium. So we show

that equilibria exist and are unique. It is convenient to change variables and write

$$y_0 := x_0 + x_1 + x_2 + x_3, \quad y_1 := x_1 + x_2 + x_3, \quad y_2 := x_2 + x_3, \quad y_3 := x_3.$$

In terms of these variables, we may set  $\dot{y}_i = 0$ ,  $i = 0, 2, 1, 3$ , so that the equilibria are precisely the solutions of:

$$\begin{aligned} \alpha_2(x_2) &= c \\ \alpha_1(x_1) &= \alpha_2(x_2) + \beta_1(x_2) \\ \alpha_0(x_0) &= \alpha_2(x_2) + \beta_0(x_1) \\ \gamma_3(x_3) &= \gamma_2(x_2). \end{aligned}$$

This shows uniqueness (all the functions are strictly increasing), and existence follows from, respectively, (7), (6), (4), and the fact that  $\gamma_3$  is unbounded. ■

## 4 Closing the Loop

Now we are ready to apply the main theorem in [2]. In order to do this, we need to plot the characteristics. See Figure 3 for the “spiderweb diagram” (the dotted and dashed curves are the characteristics) that shows convergence of the discrete iteration described in [2] when we pick the parameter  $v_s = 0.4$ . The theorem implies that no oscillations can happen in that case, even under arbitrary delays in the feedback from  $P_N$  to  $M$ .

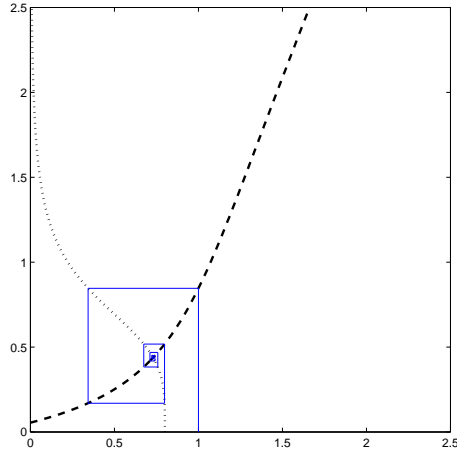


Figure 3: Stability of spiderweb ( $v_s = 0.4$ )

On the other hand, for a larger value, such as  $v_s = 0.5$ , the discrete iteration conditions are violated; see Figure 4 for the “spiderweb diagram” that shows

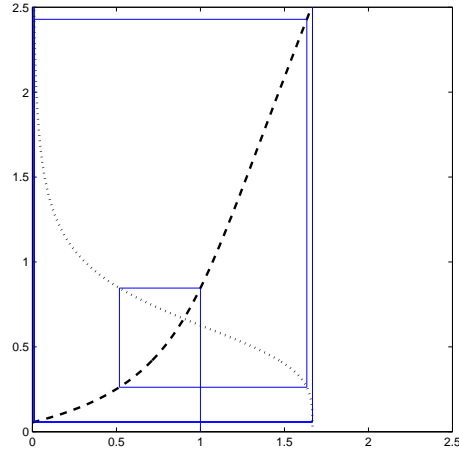


Figure 4: Instability of spiderweb ( $v_s = 0.5$ )

divergence of the discrete iteration. Thus, and one may expect periodic orbits in this case. Indeed, simulations show that, for large enough delays, such periodic orbits arise, see Figure 5.

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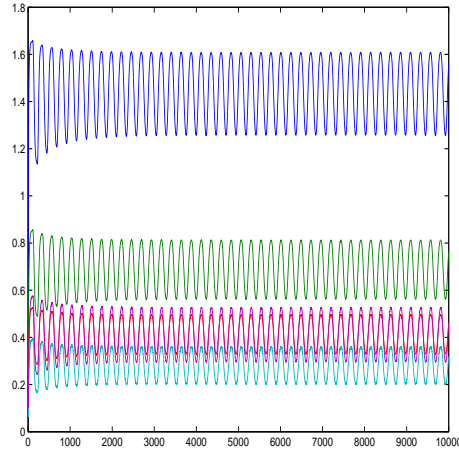


Figure 5: Oscillations seen in simulations ( $v_s = 0.5$ , delay of 100, initial conditions all at 0.2), using MATLAB's dde23 package

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